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TRANSPLENIC PASSAGE OF TUMOUR CELL EMBOLI

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(Received September 23, 1953)

One of the most actual tasks of modern oncology is to recognize the mutual effect of tumour and organism [13]. This complex of problems comprises among others, the mechanism responsible for the origin and distribution of tumour metastases.

It is a well known fact that tumour metastases in the spleen occur but infrequently [1, 7, 9, 25 etc.]. *Yokohata* (1927), studying serial sections of the spleen, could demonstrate microscopic metastases in 34 per cent of subjects dying with cancer. In the autopsy material of this department 11 macroscopic splenic metastases by way of the blood stream were found among 940 carcinomas encountered in 13.300 autopsies [10], an incidence of 1.17 per cent. On the basis of similar observations and certain animal experiments the possibility of a tumour inhibitory, defensive function of the spleen had long been raised [2, 6, 7, 12, 18], but it can still not be considered as clarified. On the other hand, the mechanistic theory of the distribution of metastatic tumours [24, 3, 19] attaches importance solely to hydrodynamical factors in the origin of splenic metastases.

In previous studies it was found that a Brown-Pearce cancer cell suspension administered intracardially into the left ventricle results in widely distributed metastases ; in the spleen, however, no metastases occurred in any of the cases. In experimental generalized carcinosis, owing to the complete lack of splenic metastases, we had to consider it an open question whether intrasplenic carcinolysis or transplenic passage of cancer cells occurs [11]. To approach this question a method has been elaborated and it is the purpose of this paper to report the details of such experiments.

Methods

To determine whether or not tumour cell emboli pass through the spleen, it seemed necessary to examine both blood from the splenic vein and a suspension of splenic tissue. It was further intended to ligate the hepatic artery, and then to inject a tumour cell suspension into the arterial blood, supposing that if the liver was completely eliminated from the arterial circulation and in spite of this metastases would occur in the liver, they can originate solely

from tumour cell emboli carried there by the portal vein. This project had, however, to be given up as elimination of the liver from the arterial circulation can hardly be realized in the rabbit.

Blood from the splenic vein has been taken from rabbits laparotomized under superficial ether anaesthesia a few seconds after 1,5 ml of a 20 per cent fresh Brown-Pearce cancer cell suspension in sterile physiological saline had been injected into the left ventricle. The tumour cell suspension was prepared from living tumour tissue of rabbits in which 18 to 20 days earlier Brown-Pearce cancer had been intratesticularly transplanted. Blood from the splenic vein was taken either by puncturing the vein with a thin cannula or by incising it, taking the greatest care not to injure the artery. The slowly trickling blood had been collected with a syringe containing 0,5 ml of a 3,8 per cent sodium citrate solution. After obtaining 2 or at most 3 ml of blood, 1 ml of of the blood-citrate mixture has been immediately injected into the vein of two (in one instance of three) other rabbits.

Immediately thereafter the spleen was removed and a 20 per cent suspension in sterile physiological saline solution prepared from it. Volumes of 0,5 ml of the suspension were injected within the shortest possible time into each of the testicles of other rabbits.

All in all 6 rabbits were injected intracardially with the tumour cell suspension. The blood from the splenic vein of these animals was intravenously injected in other 13 rabbits, while further 9 rabbits were given the spleen suspension.

Results

The fate of the 13 rabbits intravenously injected with venous blood which passed through the spleen of other rabbits into the left ventricle of which a tumour cell suspension had been injected, was as follows. One rabbit died spontaneously after two, another one after threemonths. In the first animal an intercurrent illness occurred and no metastases could be detected at autopsy, while in the other numerous and widely distributed metastases were revealed. The remaining 11 rabbits were sacrificed 30 days after they had been injected with blood from the splenic vein. Metastases occurred in the organs of eight rabbits ; in three animals no metastases could be ascertained. Accordingly, injection of venous blood which had passed the spleen led to tumour formation in 9 out of 13 rabbits (Table I).

TABLE I

Group	Number of rabbits	Cancer produced
Rabbits injected with blood of splenic vein	13	9
Rabbits injected with spleen suspension	9	1

From among the 9 rabbits treated intratesticularly with spleen suspension, one died spontaneously after 19 days, another after 6 weeks and a third one after 8 weeks. Tumour developed only in the first animal, in the testicle of which viable Brown-Pearce cancer tissue could be found but no metastases were formed. The other six rabbits were killed 30 days after injecting the spleen

suspension. Macroscopic tumour tissue could be found neither in the testes, nor in other organs. Both testes of all animals were subjected also to microscopic examination but no viable tumour tissue could be demonstrated in any of them. Accordingly, after intratesticular administration of the spleen suspension, tumour resulted only in one out of 9 rabbits.

TABLE II

Distribution of metastases in rabbits injected intravenously with blood of the splenic vein

Number of rabbits	Lung	Liver	Kidney	Adrenal	Spleen	Brain
9	7	5	7	5	1	1

Distribution of tumours in rabbits which had received intravenously blood from the splenic vein is shown in Table II. Tumours were developed most frequently in the lungs and kidneys (7 cases), next in the liver and adrenals (5 cases), in the omentum (2 cases), and finally in the brain, spleen, ovaries, subcutaneous connective tissue and the calvarium (1—1 cases). The metastases were multiple, mainly those in the lungs, kidneys and liver. In these organs a great number of tumour nodes, ranging in size from pinhead to hazelnut, even walnut, could be found (Fig. 1—4.)

It is noteworthy that in two rabbits there was no tumour detectable in the lungs, not even microscopically, although numerous tumours appeared in the other organs.

Discussion

On the basis of the peculiar »indolence« of the spleen toward malignant tumours, as has already been mentioned, several authors have suggested that that organ had an antiblastic property, while others ascribe the rarity of splenic metastases to purely mechanical factors, i. e. to the special circulatory conditions of the spleen. In *Sappington*'s (1922) opinion, the acute-angled deviation of the splenic artery at the site where it originates from the coeliac artery must be made responsible for the allegedly rare entree of tumour cell emboli into the spleen. This explanation is, however, inadequate, the spleen being one of the most frequent sites of thromboembolism. According to *Kettle* (1912), it is the rhythmic contraction of the spleen which prevents the lodgement of tumour cells. This view is not acceptable either, considering that the lung, despite its extensive movements, is one of the most frequent sites of metastases. Some attribute a role to the lack of afferent lymph vessels [20, 4]. This, however, may be applied also to other inner organs, since lymph nodes alone possess afferent lymph vessels.

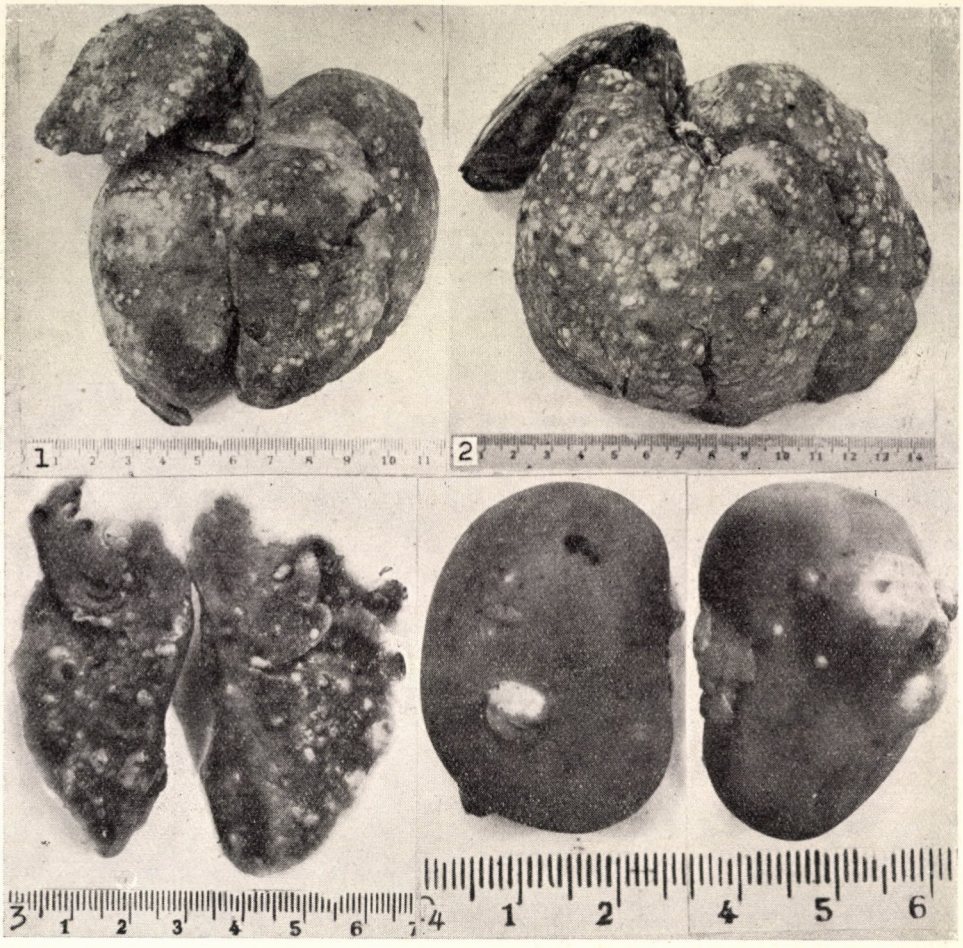

Fig. 1. Numerous tumourous nodes in the liver of a rabbit treated intravenously with blood taken from the splenic vein of a rabbit into the left ventricle of which a cancer cell suspension had been injected. Killed 30 days after intravenous injection.

Fig. 2. Same treatment as in Fig. 1. Liver showing numerous tumourous nodes.

Fig. 3. Same treatment as in Fig. 1. Large number of tumourous nodes in the lung. Same animal as in Fig. 2.

Fig. 4. Same treatment as in Fig. 1. Tumourous nodes in the kidneys.

Coman et al. (1951) observed, shortly after injecting a Brown-Pearce cancer cell suspension into the left ventricle, tumour cells in the spleen, though in a smaller number than in other organs. The tumour cells were lodged mainly in the thick-walled arterioles. The uncommon occurrence of metastases in the spleen is explained in that way that the tumour cells cannot leave the arterioles and presumably they do not survive.

Walther's conception as to the dissemination by the blood stream, according to which the distribution of metastases is determined solely by hydrodynamical factors and the primary and secondary filters (lung and liver) comprised in the circulation, has been criticized lately by several authors. *Fanfani* et al. (1951) object with reason to the omission of certain biological and functional factors. Soviet authors emphasize the role of the nervous system in the origin of metastases. The experimental data of *Shabad* (1930) and *Klenitsky* (1938) support the assumption that formation of metastases is not an autonomous process but one depending on the state of the whole organism. *Petrova* (1946), on the basis of her observations, has attributed the for mation of tumours of different character and localization to functional lesions of the cerebral cortex. A long series of investigations has been carried out in *Speransky*'s institute to examine the role of the nervous system in the mechanism of metastasis formation. *Lebedinskaia* and *Soloviev* (1951) have concluded from their experiments that the localization of metastases cannot be explained but with the permanent trophical reflexes arising from the tumour, and further, that in that mechanism reflexes and receptors of the vessels have without doubt their part.

It is thought that the results of our experiments reported suffice to support a definite view. The fact that intravenous injection of 1 ml of blood taken from the splenic vein after injecting a cancer cell suspension into the left ventricle has sufficed to produce generalized carcinosis in most of the rabbits thus treated, proves that a large number of tumour cells had to pass through the spleen. The findings prove not only the transplenic passage of tumour cell emboli, but suggest a transpulmonary passage as well. In two out of 13 rabbits treated with blood from the splenic vein tumours were namely formed in several organs, in spite of the fact that no tumour developed in the lungs. This confirms the pertaining statements of *Zeidman* and *Buss* (1952).

The eventual argument that the transplenic passage were due to narcosis or abdominal manipulation, must be repudiated. In previous experiments [11] the tumour cell suspension had been injected without narcosis or any other manipulation into the left ventricle of 20 rabbits, and still no metastasis occurred in the spleen of any of the animals, in spite of the fact that 15 of them died with generalized carcinosis.

In connection with our statement that no tumour could be induced with a suspension made from the spleen removed a few minutes after a tumour cell

suspension had been injected intracardially, the objection may be raised that this is merely a sign of the earlier suggested tumour inhibitory effect of the spleen. This is, however, contradicted by the following experiment. ½ ml of a cancer cell suspension was injected under the splenic capsule of six rabbits. Three of the animals died in 10 to 26 days, the others were killed on the 33rd day. Tumour was formed in the spleen of all the six rabbits. *Goldmann* had stated already in 1911 that a tumour transplanted into the spleen grows just as in any other organ.

On the basis of the above results *it would seem necessary to revise our concepts concerning the rarity of splenic metastases. The rarity of metastatic carcinoma in the spleen is due to the fact that in general the spleen does not retain the tumour cells carried there by the blood stream.* Should this hold true, then inhibition or impediment of the transplenic cell passage must result in tumour formation in the spleen. To prove this, the following experiment has been carried out. Venous congestion was produced in one half of the spleen by ligating some small branches of the splenic vein. 6 days after this intervention, 1,5 ml of a 20 per cent cancer cell suspension were intracardially injected into the left ventricle. Tumour developed in the spleen but only in that part of the organ of which the small veins had been ligated.

The question is whether present knowledge concerning the blood circulation of the human spleen permits of assuming the transplenic passage in man. *Kádár* (1951) demonstrated with the microcorrosion method that the arterioles of the spleen open directly in the sinuses, the space of which communicates with the pulpa, emphasizing, however, that the actual communication between sinus and pulpa spaces depends on the size of the openings in the sinus, which are regulated partly by nervous and partly by humoral factors. The anatomical investigations of *Kádár* support in every respect the transplenic passage of tumour cell emboli demonstrated in our own experiments. In this process the part played by the nervous system is most significant; it regulates by way of the vascular receptors the communication between sinus and pulpa spaces, making it possible that tumour cells carried by the blood should avoid the pulpa spaces and pass immediately from one system into the other.

Summary

Experiments were performed with Brown-Pearce rabbit cancer in order to approach the cause of the well-known infrequency of metastatic carcinoma in the spleen. Blood taken from the splenic vein of rabbits after intracardial (left ventricle) injection of a cancer cell suspension was intravenously injected to other rabbits. Generalized carcinosis was thus produced in 9 out of 13 rabbits. The spleen suspension of the intracardially treated rabbits has been injected into the testicles of 9 other animals. A tumour was subsequently found in the testis of only one animal.

It is concluded that tumour cell emboli are able to pass through the spleen. The finding suggests also the existence of a transpulmonary passage beside the transplenic one. These mechanisms may play an important role in the distribution of cancer metastases. The nervous system has a considerable part in this process; its effect is supposed to consist in influencing the permeability of the filters and consequently the possibility of the tumour cell passage.

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ПРОХОД ЭМБОЛОВ, СОСТОЯЩИХ ИЗ ОПУХОЛЕВЫХ КЛЕТОК, ЧЕРЕЗ СЕЛЕЗЕНКУ

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Резюме

Целью авторов было, путем опытов над кроликами, применяя рак Браун— Пирса, выявить причину того общеизвестного факта, что метастазы опухолей в селезенке являются редкостью. Кровь, взятая из селезеночной вены кроликов, которым предварительно прививали внутрисердечным путем (в левый желудочек) взвесь раковых клеток, была впрыснута другим кроликам внутривенно. Среди 13 обработанных таким образом кроликов, у 9 животных развился общий карциноз. Затем авторы прививали взвесь, изготовленную из селезенки кроликов, привитых внутрисердечно раковыми клетками, в яички 9 других кроликов. Однако, в данном случае опухоль развивалась в яичке лишь одного животного.

Достигнутые авторами результаты доказывают, что состоящие из опухолевых клеток эмболии могут проходить через селезенку. Согласно данным исследований следует предполагать, что кроме прохода через селезенку существует также проход через легкие. По мнению авторов эти механизмы играют важную роль в локализации раковых метастазов. Авторы считают, что в этом процессе большое значение следует приписать нервной системе, действие которой, по их мнению, состоит в том, что она влияет на проходимость фильтров, и тем самым и на возможность прохода опухолевых клеток.